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Simultaneous Quantitative Determination of Formoterol Fumarate and Fluticasone Propionate by Validated Reversed-Phase HPLC Method in Metered dose inhaler

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ABSTRACT

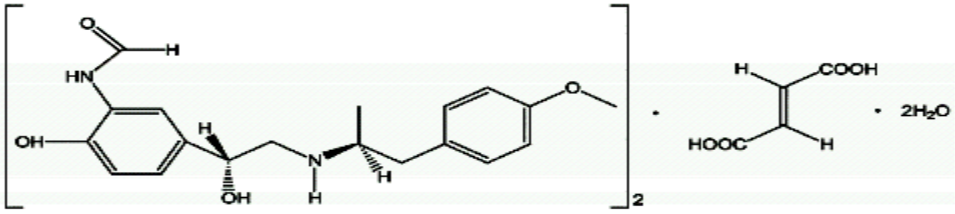
A simple and rapid HPLC method was described for the simultaneous determination of Formoterol fumarate and Fluticasone propionate in Metered Dose Inhaler formulation. The assay involved an isocratic elution of these two component on water spherisorb C_8 column (15 cm X 4.6 mm, 5 µm) using a mobile phase composition of Buffer: Acetonitrile: methanol and pH adjusted to 3.0 with dilute orthophosphoric acid. The flow rate was 1.5 mL min^{-1} and the analytes monitored at 215 nm. Separation was completed within 15 min. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 0.41 to 0.85 µg mL^{-1} for Formoterol fumarate and 6.0 to 37 µg mL^{-1} for Fluticasone propionate. The limits of detection (LOD) were found to be 0.048 µg mL^{-1} and 0.05 µg mL^{-1} for Formoterol fumarate and Fluticasone propionate respectively. All the validation parameters were with in the acceptance range according to ICH norms. Developed method was rapid and convenient, which could be successfully applied for the routine control of both the component.

Key words: Formoterol fumarate, Metered dose inhaler, chronic obstructive pulmonary disease.

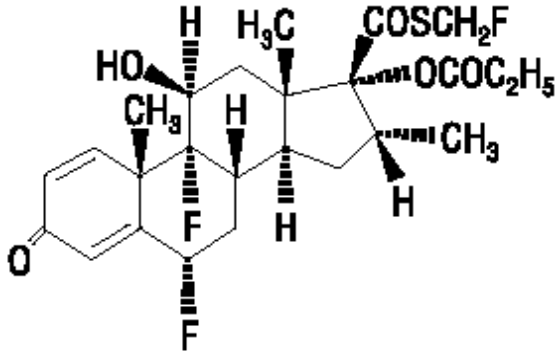
INTRODUCTION

At present it is estimated that Hundreds of million of people suffer from chronic respiratory diseases worldwide. Drug delivery by the inhalation route is a rapidly developing and challenging aspect of pharmaceutical product development [1-2]. Inhalation drugs, in the forms of nasal sprays, metered-dose inhalers (MDI), dry powder inhalers (DPI), and nebulizers, are traditionally used for treatment of asthma and COPD [3]. For prolonged duration of action, more effectiveness and quick relief most of the inhaler products has been launched in composition like β_2-adrenorecptor with corticosteroids to meet the market demand [4]. A significant synergistic therapeutic benefit can be obtained in the treatment of inflammatory or obstructive airways diseases by using a composition containing Formoterol, or a salt or solvate thereof, and

Fluticasone propionate in the form of inhaler product. Formoterol fumarate (Fig.1a) is a long-acting selective β2-adrenergic receptor agonist (β2-agonist). Inhaled Formoterol fumarate acts locally in the lung as a bronchodilator [5-6]. Formoterol appear to be more effective than shorter-acting β_2-agonist in the treatment of nocturnal and exercise induced asthma [7-8]. Fluticasone propionate (Fig.1b) is a highly potent, 2nd generation trifluorinated glucocorticosteroid based on the androstane nucleus. It is used in the treatment of asthma by inhalation and allergic rhinitis intranasally. Fluticasone propionate has high



(a)



(b)

Fig. 1. Chemical structure of Formoterol fumarate (a) and Fluticasone propionate (b).

binding affinity with glucocorticoid receptors (GR). The results include alteration of transcription and protein synthesis, a decreased release of leukocytic acid, prevention of macrophage accumulation at inflamed sites, reduction of collagen deposition, inhibition of histamine and kinin release [9-10]. To ensure the quality of inhalation drugs simple and economic analytical methods need to be developed. Literature survey did not reveal any reported method for the analysis of Formoterol fumarate and Fluticasone propionate simultaneously in any type of formulation. But various analytical methods for quantitative determination of single component or in combination with any other components has been described in literature like determination of Formoterol in rat plasma by HPLC [11], in human plasma by LC-MS [12], determination of a process impurity in Formoterol fumarate by gas chromatographic method [13], electrochemical detection[14], RP-HPLC method [5], radioimmunoassay [15] and for determination of Fluticasone; by RP-HPLC method in combination with salmetrol [16], by LC-MS method [17] and in human plasma by LC-MS method in combination with budesonide [18].

MATERIALS AND METHODS

Chemicals and Materials: Samples of Formoterol fumarate and Fluticasone propionate were obtained from VAMSI Labs limited (Solapur, MH, India) and Arch Pharma Labs limited (Thane, MH, India) respectively and used as received. Acetonitrile (HPLC grade), Methanol (HPLC grade) and analytical grade orthophosphoric acid were purchased from E-Merck and

Spectrochem limited (Mumbai, India) respectively. In-house purified water (USPgrade) was used throughout the study.

Standard Solutions:
A standard solution of Formoterol fumarate and Fluticasone propionate at the target concentration of 0.6 µg and 25 µg mL^{-1} respectively were chosen for this study.

Preparation of diluent:
A filtered and degassed mixture of water, acetonitrile and methanol was prepared in the ratio of 300:350:350 respectively.

Solution A: Stock solution for Formoterol fumarate:
Accurately weighed about 37.5 mg of Formoterol fumarate working standard was transferred to 50 mL volumetric flask containing about 25 mL of diluent and the solution was sonicated for 10 min or until the solid completely dissolved keeping the water in the sonicator at ambient temperature. Then the volumetric flask was filled to the mark with diluent. A 2.0 mL portion of the resulting solution was then transferred into a 100 mL volumetric flask filled to volume with diluent and mixed thoroughly.

Solution B: Stock solution for Fluticasone propionate:
Accurately weighed about 62.5 mg of Fluticasone propionate working standard was transferred to 100 mL volumetric flask containing about 50 mL of diluent and the solution was sonicated for 10 min or until the solid completely dissolved keeping the water in the sonicator at ambient temperature. Then the volumetric flask was filled to the mark with diluent and mixed thoroughly. 4.0 mL of stock solution A and solution B was transferred in to 100 mL volumetric flask. Then make up the volume up to the mark with diluent and mix thoroughly. Here final target concentration of Formoterol fumarate and Fluticasone propionate were 0.6 µg and 25 µg respectively.

Test Solution Preparation for MDI:
Removed the pressurized two canisters from the actuator and placed each canister in plastic bag in upright position chilled it to -20^0C for 30 min and then carefully pierced a small hole on the shoulder of each canister. Allowed the propellants to evaporate and removed the top. Now the top and valve of the opened canister was washed with diluent. Then 10 mL of diluent was added in each canister and sonicated to dissolve at ambient temperature. Now the content of each canister were transferred to 100 mL volumetric flask. Further repeat above procedure to 2 x 10 mL of diluent and sonicated to until dissolved. Both the canisters again rinsed with diluent. Then volumetric flask was filled up to the mark with diluent and mixed. A 10.0 mL portion of this resulting solution was diluted up to 100 mL with diluent and mixed thoroughly.

Instrumentation:
The chromatographic separations were performed using Shimadzu LC 2010C integrated system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS VP software. The mobile phase consists of Buffer (Ammonium di-hydrogen orthophosphate; pH-3.0): Acetonitrile: methanol in the ratio 450:300:250 v/v, filtered through a nylon membrane and degassed under vacuum before use. The water spherisorb C_8 column (15 cm X 4.6 mm), 5 µm was used as a stationary phase. The analytes were monitored with UV detection at 215 nm. Unless stated otherwise, all separations were performed at ambient temperature using a 1.5 mL min^{-1} flow rate, a 50 µL

injection volume, and a 15 min run time. The system suitability parameters displayed in Table 1 were evaluated throughout the study.

Table 1 System suitability

Parameter	Acceptance criteria	Result
% R.S.D. for peak areas in five standard injection	NMT 3.0%	Less than 1%
Resolution (Formoterol fumarate/ Fluticasone propionate)	NLT 1.5	4.6
Tailing factor	NMT 2.0	1.04 & 1.09 for both components

Method Development

Prior to chromatographic method development, the detection wavelength was determined by obtaining the UV spectra of solutions of both the drugs. As expected, both the analytes show maxima absorbance at 215 and 236 nm for Formoterol and Fluticasone respectively. From the spectra obtained, lowest wavelength detection i.e. 215 nm was chosen in order to achieve a good sensitivity for simultaneous determination of both the analytes. The chromatographic separations of Formoterol fumarate and Fluticasone propionate were investigated at 215 nm wavelength using different mobile phases consisting of di-sodium hydrogen phosphate, potassium hydrogen phosphate and/or acetate buffers in combination with methanol or acetonitrile on different analytical C_{18} columns. The separation of the analytes varied substantially with the chromatographic conditions examined. For instance, a composition of 60:40 v/v of buffer solution (KH_2PO_4 ,pH-6): acetonitrile produced no clear resolution between peaks of Formoterol and Fluticasone propionate. A trial with isocratic elution using a mobile phase consisting of ammonium acetate buffer (pH-5.0) and methanol in the ratio 65:35 on Phenomenax Luna C_{18} column did not produce good separation; only single peak of Formoterol fumarate was obtained. Finally, a mobile phase consisting of ammonium di-hydrogen phosphate (pH-3.0), acetonitrile and methanol were used in the ratio 35:25: 40 respectively on the column water spherisorb ODS (25 cm X 4.6 µm), 5 µm. Both the compounds were detected having some noise in base line and also it was found that propellant peak of MDI test solution was interfering with the peak of Formoterol. Now the ratio of mobile phase was optimized as ammonium di-hydrogen orthophosphate (pH-3.0), ACN, and methanol (450: 300:250), it offered a good separation of both the analytes at ambient temperature on the column water spherisorb ODS (15 cm X 4.6 µm), 5 µm. Under these conditions, and using a flow rate of 1.5 mL min^{-1} and a run time of 15 min, Formoterol elutes at about 7.0 min and Fluticasone at about 9 min approx (Fig 2).

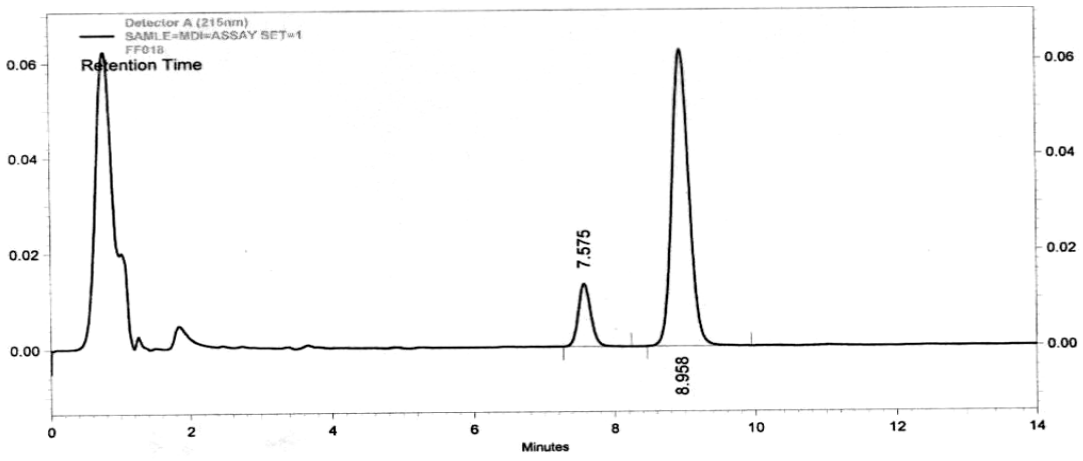
Method Validation

Linearity:

The plot of peak area responses against concentration is shown in Fig 3 and 4. It can be seen that plot is linear over the concentration range of 0.4 to 0.8 µg mL^{-1} and 6 to 37 µg mL^{-1} for Formoterol fumarate and Fluticasone propionate respectively with a correlation coefficient (r^2) 0.9999.

Quantitation Limit (QL) and Detection Limit (DL):

The detection limit of Formoterol fumarate and Fluticasone propionate were found to be 0.048 µg mL^{-1} and 0.05 µg mL^{-1} respectively. The % RSD was found to be less than 5.0% for five set of LOQ solution for both the components.



Detector A (215nm) Pk #	Name	Retention Time	Area	Resolution	Asymmetry
1	Formoterol Fumarate	7.58	161070	0.00	1.09
2	Fluticasone propionae	8.96	1036777	3.61	1.04

Fig.2. Chromatogram for Test Solution of MDI

Accuracy/recovery:

The data presented in Table 2 show excellent recoveries for metered dose inhaler at all levels. The average recoveries for triplicate determinations at 20, 100, and 150% levels for Formoterol fumarate were 103.4, 103.1 and 103.0%, with R.S.D. of 1.2, 1.5 and 1.3%, respectively and for Fluticasone propionate were 103.2, 100.2 and 100.9%, with R.S.D. of 1.1, 0.2 and 0.4%, respectively.The R.S.D. value for overall mean recovery for Formoterol fumarate and Fluticasone propionate were found to be 1.3% and 0.5% respectively.

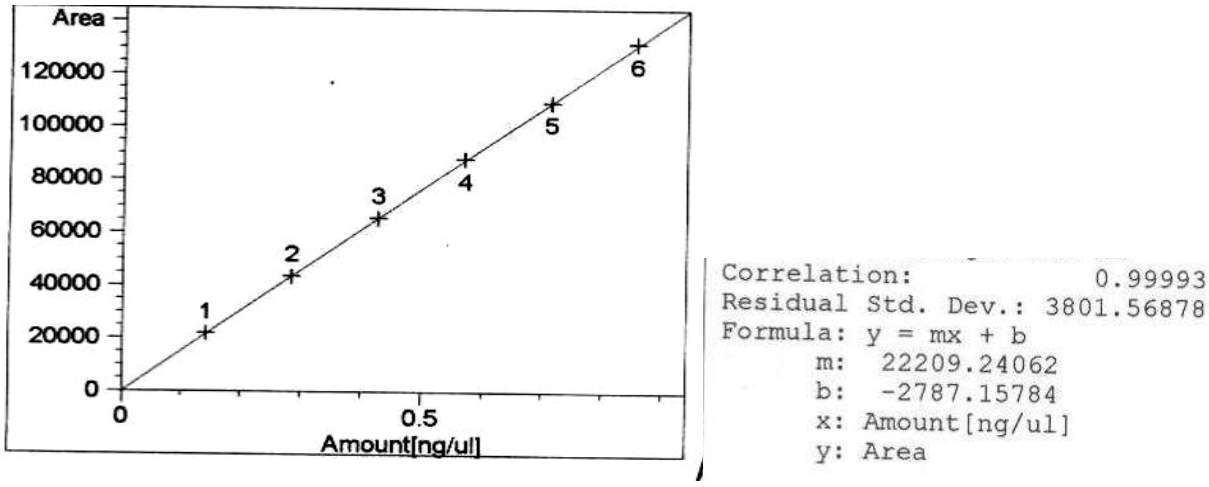


Fig.3. Linearity plot for Formoterol fumarate

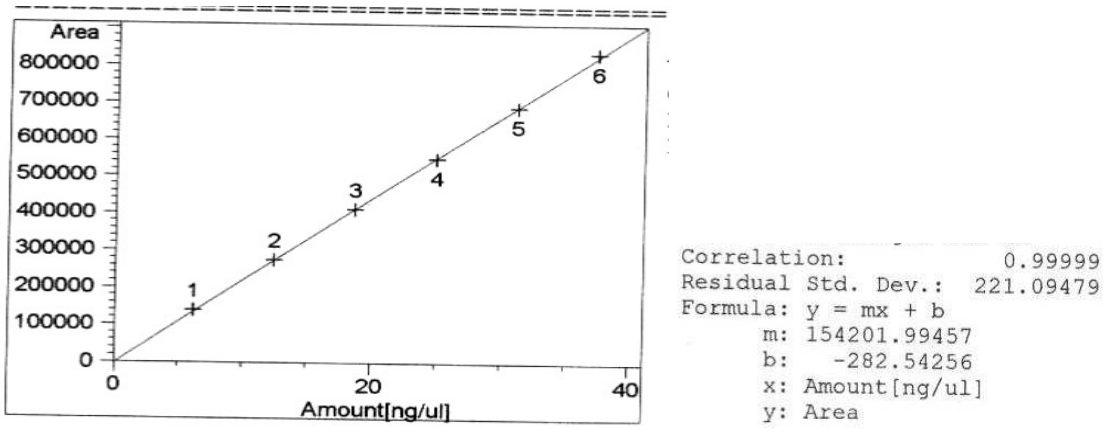


Fig.4. Linearity plot for Fluticasone propionate

Table 2 Accuracy/recovery

Level (%)	Actual amount(mg)	Recovered amount(mg)	% recovery Mean (n=3)	% R.S.D.
For Formoterol fumarate				
20%	13.80	14.20	103.4	1.2%
100%	69.00	71.15	103.1	1.5%
150%	103.50	105.78	103.0	1.3%
For Fluticasone propionate				
20%	650.00	670.39	103.2	1.1
100%	3250.00	3255.70	100.2	0.2
150%	4875.00	4919.60	100.9	0.4

Excellent recovery which was found to be within the range of 93.0% to 107.0% at each level and low R.S.D. value showed that the method is suitably accurate for potency assay of Formoterol fumarate and Fluticasone propionate in the MDI formulation.

Precision:

The R.S.D. of peak area responses for five replicate injections was found to be less than 3.0%, which met the acceptance criterion established for the method.

Table 3 Repeatability/intermediate precision of the assay method

Sample	Analyst 1, day 1		Analyst 2, day 2	
	%Formoterol	%Fluticasone	%Formoterol	%Fluticasone
1	142.22	156.41	142.00	155.97
2	140.56	154.23	141.61	155.38
3	141.18	155.42	141.21	154.43
4	141.16	156.71	142.10	156.32
5	141.40	156.46	141.55	155.51
Mean	141.30	155.84	141.69	155.52
%R.S.D.	1.0	1.1	0.3	0.5
Grand mean	141.49	155.68		
%R.S.D.	0.65	0.41		

The R.S.D. value for intraday precision of the method was 1.0% and 1.1% for Formoterol and Fluticasone respectively. The R.S.D. value for intermediate precision performed by a second analyst on different day using a different instrument was 0.3% and 0.5% for Formoterol and Fluticasone respectively. It was found that there is no significant difference between the intraday

and intermediate grand mean values (Table 3), thus the method is suitably precise and reproducible.

Specificity:

Though not shown in this report for the sake of brevity, the chromatogram demonstrates that there is no interference of diluent and placebo with spiked API. Peak purity was also found to be not less than 0.990 for both the components. Well separation of both the analytes having the resolution more than three and also good peak shape indicates that the method is Specific and selective for its intended purpose.

Table 4 Method robustness

Compound	Parameter changed	% RSD in Normal and Changed condition (n=5)		
	Temperature	% RSD Normal	% RSD (-5°C)	% RSD (+5°C)
Formoterol		0.03	0.21	0.07
Fluticasone		0.05	0.05	0.20
	pH	% RSD Normal	% RSD (-0.2 unit)	% RSD (+0.2 unit)
Formoterol		0.15	0.18	0.15
Fluticasone		0.03	0.07	0.07
	Flow Rate	% RSD Normal	% RSD (-10%)	% RSD (+10%)
Formoterol		0.19	0.32	0.24
Fluticasone		0.15	0.12	0.16
	Mobile phase (methanol ratio)	% RSD Normal	% RSD (-2%)	% RSD (+2%)
Formoterol		0.30	0.41	0.18
Fluticasone		0.11	0.06	0.23

Robustness:

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance (Table 4). As expected, the retention time of the analytes decreased with increasing mobile phase flow rate and vice versa. A slight decrease in retention factor (k) of the analytes was observed with increasing column oven temperature. Changes in pH of the buffer solution did not alter the chromatographic profile of the sample components, which remained constant with 0.2 U increase or decrease in pH from the normal experimental condition. As expected, increasing the methanol content of the mobile phase proportionally decreased the retention time of the analytes, and vice versa when the methanol concentration was decreased.

Stability of standard and sample solution:

The stability of standard and sample solution of the drug substance was examined by analyzing the solutions stored at room temperature for 36 hrs. Both the solutions did not show any change in the concentration of the analyte after the storage period. The % deviation of analyte peak area was calculated from initial for both standard and sample solution which was found to be below 2.0% for both the components.

RESULTS AND DISCUSSION

The assay involved an isocratic elution of these two component on water spherisorb C_8 column (15 cm X 4.6 mm, 5 µm) using a mobile phase composition of Buffer: Acetonitrile: methanol and pH adjusted to 3.0 with dilute orthophosphoric acid. The flow rate was 1.5 mL min^{-1} and the analytes monitored at 215 nm. Separation was completed within 15 min. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 0.41 to 0.85 µg mL^{-1} for Formoterol fumarate and 6.0 to 37 µg mL^{-1} for Fluticasone propionate. The

limits of detection (LOD) were found to be 0.048 µg mL^{-1} and 0.05 µg mL^{-1} for Formoterol fumarate and Fluticasone propionate respectively.

CONCLUSION

An isocratic liquid chromatographic method has been described and validated for qualitative and quantitative determination of Formoterol fumarate and Fluticasone propionate in the metered dose inhaler formulation. Acceptable assay precision (< 3.0% R.S.D.) and accuracy (< 5.0% R.S.D.) were obtained at 20- 150% of the analytical concentration of Formoterol fumarate and Fluticasone propionate at the target concentration of 0.6 µg and 25 µg mL^{-1} and excellent linearity was achieved over a range of 0.4 to 0.8 µg mL^{-1} and 6 to 37 µg mL^{-1} for Formoterol fumarate and Fluticasone propionate respectively. The proposed HPLC method proved reliable in addition to its high sensitivity and robustness. The validation and application of this method can be adopted for potency assay of Formoterol fumarate and Fluticasone propionate in pharmaceutical dosage forms for routine analysis.

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Note: MDI formulation contains 20% and 30% overages for Formoterol fumarate and Fluticasone propionate respectively and also having total 20 extra doses.